



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification⁵ : C12N 5/20, C12P 21/08 C07K 17/14, 15/00, A61K 35/28 G01N 33/577	A1	(11) International Publication Number: WO 90/10692 (43) International Publication Date: 20 September 1990 (20.09.90)
(21) International Application Number: PCT/US90/01392 (22) International Filing Date: 14 March 1990 (14.03.90) (30) Priority data: 323,646 15 March 1989 (15.03.89) US (71) Applicant: UNIVERSITY OF FLORIDA [US/US]; 223 Grinter Hall, Gainesville, FL 32611 (US). (72) Inventor: CASSANO, William, F. ; 2635 North West 28th Place, Gainesville, FL 32605 (US). (74) Agents: SALIWANCHIK, David, R. et al.; Saliwanchik & Saliwanchik, 2421 N.W. 41st Street, Suite A-1, Gaines- ville, FL 32606 (US).		(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European pa- tent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: MONOCLONAL ANTIBODY FOR USE IN DETECTION AND TREATMENT OF CHILDHOOD LEUKEMIA (57) Abstract The subject invention pertains to a novel cell line and the monoclonal antibody produced by the cell line. Advantageously, the novel monoclonal antibody binds specifically with the acute lymphocytic leukemia antigen. Thus, this antibody can be used in the detection of childhood leukemia as well as in therapeutic procedures such as bone marrow purging. The novel monoclonal antibody can also be used to construct hybrid toxin proteins which can selectively seek out and eliminate target cells.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MR	Mauritania
BE	Belgium	GA	Gabon	MW	Malawi
BF	Burkina Faso	GB	United Kingdom	NL	Netherlands
BG	Bulgaria	HU	Hungary	NO	Norway
BJ	Benin	IT	Italy	RO	Romania
BR	Brazil	JP	Japan	SD	Sudan
CA	Canada	KP	Democratic People's Republic of Korea	SE	Sweden
CF	Central African Republic	KR	Republic of Korea	SN	Senegal
CG	Congo	LI	Liechtenstein	SU	Soviet Union
CH	Switzerland	LK	Sri Lanka	TD	Chad
CM	Cameroon	LU	Luxembourg	TG	Togo
DE	Germany, Federal Republic of	MC	Monaco	US	United States of America
DK	Denmark				

DESCRIPTIONMONOCLONAL ANTIBODY FOR USE IN DETECTION
AND TREATMENT OF CHILDHOOD LEUKEMIA

5

Background of the Invention

Autologous bone marrow transplantation (ABMT) following high dose chemotherapy and radiotherapy has been recently used in many medical centers to treat different forms of poor prognostic cancer (Bone marrow autotransplantation in man. Report of an international study. Lancet i:960, 1986). A number of procedures have been employed to purge the marrow sample of tumor cells (Proceedings of the First International Workshop on Bone Marrow Purging. Bone Marrow Transplant 2 (Supp. 2), 1987). Although the depletion of tumor cells from bone marrow for autologous transplantation has been attempted by a variety of methods (Kemshead, J.T., L. Heath, F.M. Gibson, F. Katz, F. Richmond, J. Treleaven, and J. Ugelstad [1986] Br. J. Cancer 54:771-778) magnetic microspheres and monoclonal antibodies have been most successful (Treleaven, J.G., F.M. Gibson, J. Ugelstad, A. Rembaum, T. Philip, G.D. Caine, and J.T. Kemshead [1984] The Lancet Jan. 14(i) (8368):70-73). This technique has been shown to be a safe, inexpensive, and reliable procedure for bone marrow purging (negative selection) (Gee, A.P. et al. [1987] Bone Marrow Transplantation 2(Supp 2)). More recently, this technology has also been tried for isolation of pure functionally active cells (positive selection) (Gaudernack, G., et al. [1986] J. Immunol. Mths. 90:179).

25

Briefly, the currently used technique involves a monoclonal antibody or a cocktail of antibodies against tumor surface proteins with which the isolated bone marrow is incubated. Monodisperse polystyrene microspheres, 4.5 um in size, with covalently attached sheep anti-mouse IgG (e.g., Dynal M450, Dynal Inc.,

Great Neck, NY) are then added to the mixture in order to bind to the tumor cell-attracted monoclonal antibodies (MoAbs) (rosette formation). The magnetic cells are removed by strong magnets and the clean bone marrow is re-injected into the patient. The effectiveness of ABMT procedures depends upon several factors including the type of beads used, how the beads are coated, and the nature of the antibodies used to coat the beads. With regard to the antibodies used, it is advantageous to have antibodies which bind only to the target cells and do not remove desirable cells. Also, the binding to the target cells must be of a nature that a high proportion of these target cells are removed.

The antibodies and mixtures of antibodies which have been used to coat magnetic microspheres used in bone marrow purging systems have had moderate success. Lack of selectivity and low rates of removal of target cells have limited the effectiveness of these procedures.

The hydrophobic surface of polystyrene beads binds proteins avidly. Consequently, a high nonspecific cell binding and low recovery of healthy cells following purging is always encountered. Low and greatly variable recovery of nucleated cells following purging of neuroblastoma cells with M450 microspheres has been reported, e.g., approximately 65% (Treleaven et al. [1984], *supra*), 38-87% (Kemshead et al. [1986], *supra*), and 50% (Kemshead, J.T., J.G. Treleaven, F.M. Gibson, J. Ugelstad, A. Rembaum, and T. Philip [1985] *Progr. Exp. Tumor Res.* 29:249-255). Recently, successful depletion of T-lymphocytes from human bone marrow (Vartdal, et al. [1987]) has been reported; however, again only 43-74% of non-T cells were recovered.

The high incidence of non-specific binding of cells not only makes this procedure relatively inefficient, it has until now been the major obstacle in attempting positive selection of individual cell types, either for research purposes or therapeutic treatment. Positive selection of CD8⁺ T cells from peripheral blood mononuclear cell suspensions was successful using a different protocol, with

the MoAb directly conjugated to the magnetic bead; however, the system lacked inhibition of the cytotoxic activity of T8 cells which was thought to be partially due to the direct binding of MoAb to the microsphere surface (Gaudernack et al. [1986]).

5 It is the general objective of the subject invention to provide a modified magnetic microsphere which facilitates the specific removal of a high percentage of target cells during ABMT procedures.

10 It is a specific objective of the invention to improve the known technique of bone marrow purging by using a novel monoclonal antibody so that cancerous cells are completely and reliably removed while nucleated and hemopoietic cell progenitors are retained for autologous transplantation.

Brief Summary of the Invention

15 The subject invention concerns a novel monoclonal antibody (MoAb), the cell line used to produce this MoAb, and magnetic microspheres coated with the novel MoAb. Advantageously, the MoAb of the subject invention recognizes the common acute lymphocytic leukemia (ALL) antigen (CALLA, CD10, Bgp100). This novel antibody is a high affinity murine IgG1, kappa (IgG1k) antibody which can be used in the diagnosis and identification of childhood leukemia.

20 The novel antibody, which has been designated WCMH15.14, can also be used in the therapy of childhood leukemia. The therapy involves immunomagnetic purging of residual leukemic cells in preparation for autologous bone marrow transplantation in children with acute lymphocytic leukemia.

25 The monoclonal antibody may also find therapeutic application in hybrid toxin systems where the MoAb is coupled with a toxin such as diphtheria or reicin. These systems selectively seek out and destroy the target cancer cells.

Detailed Description of the Invention

The monoclonal antibody of the subject invention is a murine IgG1k antibody produced from the fusion of mouse spleen oocytes immunized with the Nalm-6 cell line and fused to the NS1 myeloma cell line. Successful hybrids were selected in HAT median and cloned by limiting dilution on thymocyte feeder layers.

The fusion which ultimately produced the MoAb possessing the advantageous features described here resulted in approximately 2000 successful hybrids. Of course, the production of hybrids is a very random event; no two hybrids are alike, and it is impossible to predict or control all the relevant characteristics of the resulting hybrids. For example, of the 2000 successful hybrids resulting from this fusion, only 80 were found to produce antibody which bound to Nalm-6. Of those 80 hybrids producing the desired antibody, less than half were found to have the desired selectivity. That is, 37 of the hybrids produced an antibody which bound to Nalm-6 but not Jurkat cells. Even in the unlikely event that a cell line produces the desired antibody and has the necessary selectivity, the cell line may be of no value if it is not stable. In fact, less than 30% of the selective antibody-producing hybrids identified here were stable. One further criteria which is an important factor in assessing the utility of a monoclonal antibody to detect is the ability of the antibody to bind with leukemic cells from a high percentage of individuals who suffer from the disease. For example, even in the unlikely event of finding an antibody which effectively binds with the leukemia cells from one patient, it is possible that the antibody may not recognize leukemia cells from other patients. In fact, it has been found that monoclonals rarely recognize leukemic cells from a broad range of leukemia patients.

Of the few hybrids which were found to be stable and produce a monoclonal antibody of the desired selectivity, one was found to be particularly

advantageous in terms of the range of patients whose cells it would bind with. This cell line and the MoAb, each designated WCMH15.14, are the subjects of this invention. Monoclonal antibodies which are immunologically equivalent to WCMH15.14 may also be used to practice the subject invention.

5 The cell line of the subject invention has been deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852 USA on March 2, 1989. The cultures were assigned the following accession numbers by the repository:

10	<u>Culture</u>	<u>Accession number</u>	<u>Deposit date</u>
	WCMH15.14	HB 10048	March 2, 1989

15 The subject culture was deposited under conditions that assure that access to the culture will be available during the pendency of this patent application to one determined by the Commissioner of Patents and Trademarks to be entitled thereto under 37 CFR 1.14 and 35 USC 122. The deposit is available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny, are filed. However, it should be understood that the availability of the deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

20 Further, the subject culture deposit will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, i.e., it will be stored with all the care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposits, and in any case, for
25 a period of at least 30 (thirty) years after the date of deposit or for the enforceable life of any patent which may issue disclosing the culture. The depositor acknowledges the duty to replace the deposit should the depository be

unable to furnish a sample when requested, due to the condition of the deposit. All restrictions on the availability to the public of the subject culture deposit will be irrevocably removed upon the granting of a patent disclosing it.

5 The monoclonal antibody produced by the deposited cell line has several uses in the detection and therapy of children with acute lymphocyte leukemia. For example, the antibody can be used in standard immunoassay procedures in order to detect the presence of leukemia antigens in a biological sample suspected of containing evidence of the disease.

10 One means by which the WCMH15.14 MoAb can be used in the treatment of leukemia patients is the coating of magnetic microbeads which are to be used in the purging of bone marrow from these patients. The characteristics of a monoclonal antibody that would be most useful for clinical use in purging marrow of residual leukemic cells prior to autologous marrow transplants for children with acute lymphoblastic leukemia would include: (1) the
15 ability of the antibody to bind to the leukemic cells in a high percentage of patients, (2) the ability of the antibody to efficiently purge leukemic cells from marrow when used with magnetic microspheres in the immunomagnetic purging procedure, and (3) the antibody should not bind to stem cells which are needed to reconstitute normal marrow function after transplantation.

20 Because the monoclonal antibody of the subject invention binds target cells selectively, and with a high degree of affinity, WCMH15.14, or immunological equivalents, can be used alone or in combination with a cocktail of other MoAbs to increase the efficiency of the bone marrow purging system. The microspheres can be used to coat the beads using standard procedures well
25 known to those skilled in the art. See, for example, Treleaven et al. (1984), supra; Kemshead et al. (1986), supra; Kvalheim, G., Ø. Fodstad, A. Pihl, K. Nustad, A. Pharo, J. Ugelstad, and S. Funderud (1987) Cancer Res. 47:846-851. The use of the novel monoclonal antibody described here can greatly enhance

the effectiveness of any of the procedures currently using MoAbs to purge biological samples.

Another procedure which utilizes the novel monoclonal antibody of the subject invention in the therapy of leukemia patients is the selective destruction of target cells with appropriate toxins. This technique involves the coupling of the novel MoAbs to a toxin so that the toxin is specifically delivered to the target cells. This procedure can be used in vivo and the antibody coupled to a number of different toxins, including poisonous lectins, ricin, abrin, modeccin, botulina, and diphtheria toxin. The construction of such a hybrid toxin could proceed, for example, according to the disclosures of United States Patent Nos. 4,675,382 (Murphy) and 4,792,447 (Uhr et al.) relating to hybrid proteins.

Materials and Methods

An eight week old Balb/C female mouse was immunized with the human pre-B cell line Nalm-6 by intraperitoneal injection of whole, live cells as follows: 3 million cells on day 1; 5 million cells on day 7; 10 million cells on day 14; and 20 million cells on day 21, day 28, and day 35. On day 38 the mouse was sacrificed, and the spleen was removed and disrupted by crushing between two frosted glass slides producing a single cell suspension of splenocytes. The 210 million splenocytes obtained were mixed with 200 million NS-1 myeloma cells harvested in logarithmic growth phase. This cell mixture was pelleted into two 50 cc sterile glass centrifuge tubes, and the supernatant was removed leaving a dry cell pellet. Forty percent polyethylene glycol in RPMI1640 adjusted to pH 8 was utilized to fuse the cells.

One milliliter of 40% polyethylene glycol was added to each tube and the pellet was gently resuspended by rotating the tube. After 5 minutes for the first tube and 8 minutes for the second tube, the polyethylene glycol was slowly diluted with RPMI1640 to a volume of 20 cc per tube. The tubes were then

centrifuged at 1000 RPM for 7 minutes and the supernatants were discarded. The cells were then resuspended in 40 milliliters per tube of media consisting of RPMI1640, 12% fetal calf serum, supplemental glutamine, pyruvate, penicillin, streptomycin, hypoxanthine, aminopterin, and thymidine. The cells were allowed to rest for one hour at 37°C in this medium.

Following this rest, the cells were plated out into four 96-well flat bottom plates with approximately 200 microliters per well. The plating was done with a 10 cc pipette to minimize shearing forces of the newly formed hybrids. The four plates were then incubated at 37°C with 5% CO₂ in air for 10 days, at which time vigorous growth in each well was apparent and beginning to exhaust the nutrient supply.

A mean of 5 clones per well was observed in these 4 plates, indicating that approximately 2000 successful hybrids had been formed in this fusion. Supernatants from each well were then screened for monoclonal antibodies that would bind to Nalm-6 cells and not to the T cell line Jurkat. Fifty microliters of supernatant were removed from each well, diluted with an equal volume of phosphate buffered saline (PBS), and 50 microliters were applied to corresponding wells in a round bottom 96-well plate containing Nalm-6 cells and another containing Jurkat cells. After a 30 minute incubation at 4°C, the cells were washed 3 times with cold PBS, reacted with a goat anti-mouse peroxidase conjugated antibody for 30 minutes, washed twice again, and the peroxidase substrates o-phenylenediamine and hydrogen peroxide were added. The brown color indicating a positive reaction identified the wells containing monoclonal antibody binding to the particular cell. The results of this screen indicated that 80 wells contained antibody reacted with Nalm-6 cells (20% of the 400 wells) and that 37 wells contained antibody that bound to Nalm-6 cells but not to Jurkat cells.

Fifteen of the better-growing and antibody-producing clones were then tested by indirect immunofluorescence for their ability to bind to leukemic cells from a patient with CALLA negative acute lymphoblastic leukemia, to granulocytes, to peripheral blood mononuclear cells, and to Nalm-6 cells. The distinctive pattern of CALLA expression or lack of expression on these cell types aided in the rapid identification of potential CD10 (anti-CALLA) antibodies. In addition, blocking experiments using a biotinylated, known anti-CALLA antibody were also performed with these antibody supernatants. These 15 clones were recloned by limiting dilution on thymocyte feeder layers over the ensuing 6 weeks to insure monoclonality. During this time, 3 of the 15 became unstable and ceased antibody production.

The remainder of the clones are stable and underwent extensive characterization with a variety of immunoassays, as well as immunoprecipitation experiments involving I-125 surface labeled Nalm-6 cells. One antibody, WCMH15.14, precipitated a 100 kilodalton surface glycoprotein, blocked the biotinylated CALLA antibody, and exhibited the characteristic cell binding distribution of other CD10 antibodies. The other 11 stable clones do not bind the common ALL antigen. The antibodies were isotyped using the Zymed mouse isotyping kit. WCMH15.14 was found to be a murine IgG1, kappa monoclonal antibody.

Following are examples which illustrate procedures, including the best mode, for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 - Binding Capacity and Selectivity of WCMH15.14

Purging experiments were conducted where magnetic microbeads were coated with a variety of monoclonal antibodies to test the ability of these antibody-coated beads to bind to the target cells and to assess the selectivity and range of the binding. Cells from the Nalm-6 pre-B cell line were used as representative leukemia cells. 1 x 10⁶ cells per well were placed in a 96-well microtiter plate and cooled to 4°C. One hundred ml of a 1:200 dilution of the selected monoclonal antibody in phosphate buffered saline (PBS) was incubated at 4°C for 30 minutes. The cells were then washed 3 times with PBS and transferred in cold PBS to a 12x75 mm plastic tube containing 4 ml of PBS. Next, 20 x 10⁶ magnetic beads coated with a sheep anti-mouse antibody (Dyna, Incorporated) were added to each tube. The tube was rotated at 4°C for 30 minutes. A magnet was placed against the outside of the tube as the tube was rotating for 5 minutes. The supernatant was removed, fresh PBS was added to the tube, and the process was repeated. The first and second supernatants were combined and the cells contained in them were counted. These cells represent the number of cells not removed by the magnetic bead purging process. The purging efficiency was calculated as the percentage of cells removed, known that one million cells were initially subjected to purging.

Magnetic microbeads coated with WCMH15.14 were found to bind effectively with CALLA cells, removing over 70% of these cells. Furthermore, the beads coated with WCMH15.14 were found to remove cells from 5 of the 6 patients tested. Thus, WCMH15.14 can be expected to have a high probability of effectively removing leukemic cells for a broad range of patients suffering from childhood leukemia. The ability to bind with leukemia cells from a high percentage of the patients tested also has important implications in terms of the antibody's use as a diagnostic tool. Because it recognizes a broad range of

leukemic cells, WCMH15.14 would be less likely to give false negatives when used to detect the presence of leukemic cells in a biological sample.

Example 2 - Use of WCMH15.14 in Cocktail with Other Monoclonal Antibodies

5 Several other well-known monoclonal antibodies that recognize malignant and normal pre-B cells have been shown to be useful in a cocktail along with WCMH15.14 to purge leukemic bone marrow. These antibodies include DU-ALL-1, a CD9 antibody, and HD37, a CD19 antibody. Other CD9 and CD19 antibodies, as well as other unclustered antibodies may also be useful in this
10 purging process in combination with WCMH15.14.

Example 3 - Use of WCMH15.14 to Phenotype Malignant Cells

 Antibody WCMH15.14 along with other CALLA antibodies have been used to phenotype malignant cells from patients with leukemia and lymphomas.
15 It has been clearly established through many published studies (see, for example, Foon, K.A., and R.F. Todd III (1986) J. Am. Soc. Hematology 68:1-31) that cell phenotype is predictive of the patient's prognosis and has been effectively utilized in stratifying patients to different treatment protocols. This process has dramatically improved the survival of all patients with leukemia. It is also known,
20 for example, that children with CALLA positive lymphoblasts have a better prognosis than those whose blast cells lack the CALLA antigen. This typing can proceed according to the procedure outlined in Foon and Todd, supra.

Table 1.

5	Monoclonal Antibody	Removal of cells (%)	Frequency of antigen expression* (%)
10	None	7	0
	BA-1	46	ND
	WC11.5	18	0
	24.1	59	ND
	2H7	11	ND
15	L243	81	ND
	WCMH15.2	19	33
	WCMH15.5	0	0
	WCMH15.7	7	16
	WCMH15.8	83	16
20	WCMH15.9	21	33
	WCMH15.10	6	16
	WCMH15.11	73	0
	WCMH15.12	15	67
	WCMH15.13	0	100
	WCMH15.14	71	89
	WCMH15.15	10	0

ND = Not done

*This measures the percentage of leukemia patients whose cells were recognized and bound to by each monoclonal antibody.

Claims

1 1. A cell line which produces a monoclonal antibody where said
2 monoclonal antibody binds to Nalm-6 cells but not to the T cell line Jurkat.

1 2. The cell line, according to claim 1, wherein said cell line has the
2 characteristics of HB 10048.

1 3. A monoclonal antibody which binds to Nalm-6 cells but not to the cell
2 line Jurkat.

1 4. The monoclonal antibody, according to claim 3, wherein said
2 monoclonal antibody is that which has been designated WCMH15.14 and is
3 produced by the cell line having the characteristics of HB 10048.

1 5. A magnetic microbead wherein said microbead is coated with a
2 monoclonal antibody which binds with Nalm-6 cells but not with the T cell line
3 Jurkat.

1 6. The magnetic microbead, according to claim 5, wherein said
2 monoclonal antibody is WCMH15.14 produced by the cell line having the
3 characteristics of HB 10048.

1 7. The magnetic microbead, according to claim 5, wherein said microbead
2 is further coated with a cocktail of additional monoclonal antibodies.

1 8. The magnetic microbead, according to claim 6, wherein said microbead
2 is further coated with a cocktail of additional monoclonal antibodies.

1 9. A hybrid toxin protein which comprises a monoclonal antibody which
2 binds to Nalm-6 cells but not to the T cell line Jurkat, together with a toxin.

1 10. The hybrid toxin protein, according to claim 9, wherein said
2 monoclonal antibody is WCMH15.14 produced by the cell line having the
3 characteristics of HB 10048.

1 11. The hybrid toxin protein, according to claim 9, wherein said toxin is
2 selected from the group consisting of poisonous lectins, ricin, abrin, modeccin,
3 botulina, and diphtheria toxin.

1 12. The hybrid toxin protein, according to claim 10, wherein said toxin is
2 selected from the group consisting of poisonous lectins, ricin, abrin, modeccin,
3 botulina, and diphtheria toxin.

1 13. In a method for purging bone marrow, wherein said method
2 comprises the use of antibody-coated magnetic microbeads to remove target cells,
3 an improvement wherein said magnetic microbeads are coated with a monoclonal
4 antibody which binds with Nalm-6 cells but not to the T cell line Jurkat, said
5 monoclonal antibody may be used singly or in combination with other antibodies.

1 14. The method, according to claim 13, wherein said monoclonal antibody
2 is WCMH15.14 produced by the cell line having the characteristics of HB 10048.

1 15. The use of the monoclonal antibody WCMH15.14 to phenotype
2 malignant cells from patients with leukemia and lymphomas.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 90/01392

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁵		
According to International Patent Classification (IPC) or to both National Classification and IPC C 12 N 5/20, C 12 P 21/08, C 07 K 17/14, C 07 K 15/00, IPC ⁵ : A 61 K 35/28, G 01 N 33/577		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
IPC ⁵	C 12 P, G 01 N	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	Proc. Natl. Acad. Sci. USA, volume 79, July 1982, (Washington, DC, US), R. Ueda et al.: "Serological analysis of cell surface antigens of null cell acute lymphocytic leukemia by mouse monoclonal antibodies", see pages 4386-4390, especially the abstract and tables 1,3,6	1-4,15
Y	--	5-8,13,14
Y	Cancer Research, volume 47, 1 February 1987, (Philadelphia, PA, US), G. Kvalheim et al.: "Elimination of B-Lymphoma cells from human bone marrow: Model experiments using monodisperse magnetic particles coated with primary monoclonal antibodies", see pages 846-851, especially the abstract and page 580, lines 38-46 (cited in the application) ./.	5-8,13,14
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁰ Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
22nd June 1990	19. 07. 90	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	Mme N. KUIPER	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, " with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	Cancer Research, volume 48, 15 August 1988, (Philadelphia, PA, US), H. Hara et al.: "Efficient transplantation of human non-T-leukemia cells into nude mice and induction of complete regression of the transplanted distinct tumors by ricin A-chain conjugates of monoclonal antibodies SN5 and SN6", see pages 4673-4680, especially the abstract --	1-4,9-12
A	Eur. J. Cancer, volume 37, 1986, (Oxford, GB), K. Sugita et al.: "Use of a cocktail of monoclonal antibodies and human complement in selective killing of acute lymphocytic leukemia cells", see pages 351-357, especially the abstract --	13,14
P,X	Experimental Hematology, volume 17, no. 8, August 1989, (New York, US), C.J. Biéva et al.: "Malignant leukemic cell separation by iron colloid immunomagnetic adsorption", see pages 914-920, especially the abstract -----	8,13,14